Hormone-evoked Calcium Release from Intracellular Stores Is a Quantal Process*

Shmuel Muallem‡‡, Stephen J. Pandol¶, and Timothy G. Beeker†

From the Laboratory of Membrane Biology, Research Institute, Cedars-Sinai Medical Center and University of California School of Medicine, Los Angeles, California 90048 and Veterans Administration, University of California School of Medicine, San Diego, California 92181

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Ca2+ mobilization by hormones, ionomycin, and inositol 1,4,5-trisphosphate (Ins-1,4,5-P3) were studied to determine whether Ca2+ release is a continuous or a quantal process. Hormone-mediated Ca2+ release occurs only during the first 2–4 s of stimulation. Stimulation of acini with a maximal hormone concentration following stimulation with a submaximal concentration resulted in free cytosolic Ca2+ concentration ([Ca2+]i) increase and 46Ca efflux. The peak [Ca2+]i increase induced by a maximal concentration of agonist was nearly constant when cells were prestimulated with a submaximal dose for 1–15 min. Submaximal hormone concentrations release only a fraction of intracellular 46Ca2+, after which intracellular Ca2+ content remains constant. The partially released stores remain depleted until cell stimulation is terminated, at which time the stores reload with Ca2+. For comparison, increasing concentrations of ionomycin resulted in increasing rates of Ca2+ release. Each ionomycin concentration released all the Ca2+ from intracellular stores. We therefore conclude that hormone-evoked Ca2+ release is a quantal rather than a continuous process. In permeabilized cells, increasing concentrations of Ins-1,4,5-P3 resulted in an increased fraction of Ca2+ release. No submaximal Ins-1,4,5-P3 concentrations were capable of releasing all the Ins-1,4,5-P3-mobilizable Ca2+. Therefore, it appears that the quantal properties of hormone-evoked Ca2+ release reflect the quantal properties of Ins-1,4,5-P3-mediated Ca2+ release from intracellular stores.

Stimulation of cells with Ca2+-mobilizing hormones results in a transient increase in free cytosolic Ca2+ concentration ([Ca2+]c) (1–8). [Ca2+]c increases due to Ca2+ release from intracellular stores and Ca2+ influx from the medium into the cells (2–4, 6–8). [Ca2+]c and 46Ca flux measurements show that in pancreatic acinar (5, 7, 9–14), as well as in other cells (1, 2, 15, 16), the initial rapid [Ca2+]c increase is largely due to Ca2+ release from intracellular stores. The biochemical events leading to Ca2+ release are thought to involve the stimulation of phospholipase C, which hydrolyses phosphatidylinositol 4,5-bisphosphate to release inositol 1,4,5-trisphosphate (Ins-1,4,5-P3) to the cytosol (17, 18). Ins-1,4,5-P3 releases Ca2+ from a nonmitochondrial, intracellular organelle by activation of an Ins-1,4,5-P3-specific Ca2+ conductive pathway (19, 20).

Hormone-stimulated Ins-1,4,5-P3 production and Ins-1,4,5-P3-mediated Ca2+ release from a nonmitochondrial pool have been demonstrated in many cell types (17, 18). It is generally believed that all agonists capable of triggering Ca2+ release from intracellular stores do so by stimulating the generation of Ins-1,4,5-P3 in the cytosol. Recently, it was shown that hormonal stimulation of pancreatic acini and platelets is followed by the appearance of both Ins-1,4,5-P3 and inositol 1,2-cyclic-4,5-trisphosphate (IP3) (21, 22). Although IP3 can release Ca2+ from an intracellular, nonmitochondrial store (23, 24), measurements of the time courses of Ins-1,4,5-P3 and IP3 generation (25) suggest that the initial and rapid [Ca2+]c increase is mediated by Ins-1,4,5-P3. IP3 may trigger some Ca2+ release at subsequent stimulation periods reflected by a second event in [Ca2+]c (26).

Little is known about the kinetic properties of the hormone- or Ins-1,4,5-P3-evoked Ca2+ release process. Rapid kinetic measurements suggest that the hormone-mediated [Ca2+]c increase occurs during the first 1–4 s of cell stimulation (15, 27). Ca2+ is then removed from the cytosol by Ca2+ pumping out of the cells (9–12, 18) and reuptake into the hormone-sensitive intracellular stores (29). As a result, [Ca2+]c is reduced to near-resting levels. The maintained high levels of cytosolic Ins-1,4,5-P3 during the stimulation period (22, 25, 30–32) implies that the membrane of the internal stores remains permeable to Ca2+ for as long as the cells are stimulated. Indeed, Ca2+ reloading of the internal stores in various cells requires the termination of cell stimulation (3, 4, 7, 8, 12, 33). Furthermore, the Ca2+ which is reincorporated into the stores during the stimulation period (6, 9–12, 28) can be rapidly exchanged with medium Ca2+ (29). These Ca2+ transport properties reflect Ca2+ transport in cells stimulated with maximal agonist concentrations. As expected, stimulation of cells with submaximal agonist concentrations results in a submaximal increase in [Ca2+]c. The maintained levels of Ca2+-releasing second messengers (22, 25, 30–32) and in turn, Ca2+ permeability of the internal stores (29) during the stimulation period, raises the question of whether Ca2+ release from intracellular stores is a continuous or a quantal process. For a continuous process, increasing agonist concentration should result in an increased rate and continuous Ca2+ release.
so that any agonist concentration is capable of completely depleting the internal stores of Ca\textsuperscript{2+}. For a quantal process each subcellular agonist concentration releases only a fraction of the internal stores, and Ca\textsuperscript{2+} release from intracellular stores is an "all or none" process.

[Ca\textsuperscript{2+}], and 46Ca efflux measurements from pancreatic acini indicate that hormone-evoked Ca\textsuperscript{2+} release from intracellular stores is a quantal process, whereas the Ca\textsuperscript{2+} ionophore ionomycin triggers a continuous process. The quantal properties of Ca\textsuperscript{2+} release can be attributed to the mode of Ins-1,4,5-P\textsubscript{3}-mediated Ca\textsuperscript{2+} release which also shows quantal behavior.

**EXPERIMENTAL PROCEDURES**

Materials—Cholecystokinin octapeptide was a gift from the Squibb Institute for Medical Research, Princeton, NJ. Soybean trypsin inhibitor, carbachol, atropine, and 2,3-diphosphoglyceric acid (PGA) were obtained from Sigma. Ins-1,4,5-P\textsubscript{3} and ionomycin were obtained from Behring Diagnostics. Purified collagenase (type CLSPA) was obtained from Cooper Biomedical. Fura 2/AM and Fura 2-tetra K\textsuperscript{+} salt were from Molecular Probes (Eugene, OR).

The incubation solution (solution A) for intact cell studies contained 10 mm Hepes (pH 7.4), 130 mM NaCl, 5 mM KCl, 1 mM CaCl\textsubscript{2}, 2 mM Ca\textsuperscript{2+}, 130 mM Na\textsubscript{2}SO\textsubscript{4}, 10 mM sodium pyruvate, 0.01% (w/v) soybean trypsin inhibitor, and was with or without 0.1% (w/v) bovine serum albumin.

Dispersed pancreatic acini were prepared from Sprague-Dawley rats using a collagenase digestion procedure previously described (34) with minor changes as previously specified (28). Acini prepared from the pancreas of one rat were suspended in 10–20 ml of solution A and either used immediately or stored on ice until use.

**Measurement of [Ca\textsuperscript{2+}]**—Acini from half a pancreas suspended in 5 ml of solution A were incubated with 2 mM Fura 2/AM for 20 min at 37 °C with continuous shaking. The acini were then washed twice with 50 ml of solution A and resuspended in 2–3 ml of solution to be used immediately. Approximately 100 μl of cell suspension was transferred to 1.9 ml of prewarmed media of desired composition, and fluorescent measurements were made while the cells were continually stirred and maintained at 37 °C. Fluorescence was measured with a Perkin-Elmer 650–40 fluorescence spectrophotometer with excitation and emission wavelengths set at 340 and 500 nm, respectively. [Ca\textsuperscript{2+}] was calculated as previously described (35) using a K\textsubscript{D} of 220 nM for Fura 2-Ca\textsuperscript{2+} dissociation constant.

**46Ca Fluxes: Intact Cells**—Acini from the pancreas of one rat were suspended in 10 ml of solution A containing 2 mM CaCl\textsubscript{2} and incubated at 37 °C under continuous shaking. The acini were incubated with 200 μM carbachol for 5 min. 46Ca (approximately 2 × 10\textsuperscript{5} cpm/ml) and 20 μM atropine were added and the incubation continued for another 5 min. The acini were collected by a 10-s centrifugation at 150 × g, and medium containing 46Ca was removed. We previously showed (36) that this procedure results in dislodging of the intracellular stores to isotonic equilibrium, and 85–90% of the cellular 46Ca is present in the hormone-mobilizable pool. As a result, a very good signal-to-noise ratio is obtained in efflux experiments. The 46Ca-labeled acini were suspended in 20 ml of prewarmed solution A containing 2 mM of unlabeled CaCl\textsubscript{2} and incubated at 37 °C under continuous shaking. At the indicated times, 1.5-ml samples were transferred to 10 ml of ice-cold, Ca\textsuperscript{2+}-free solution A containing 1 mM LaCl\textsubscript{3} (stop solution). The acini were then collected by a 30-s centrifugation at 150 × g and washed twice more with the same solution. The pelletized acini were dissolved by heating at 60 °C in 1 ml of 1 M NaOH, and 46Ca was counted by liquid scintillation counting.

**46Ca Fluxes: Permeable Cells**—Acini from one rat were washed twice and resuspended in 3–5 ml of a solution containing 120 mM KCl, 3 mM MgCl\textsubscript{2}, 10 mM Hepes-Na (pH 7.2 at 37 °C), 0.01 mM CaCl\textsubscript{2} (uptake medium). To adjust the free Ca\textsuperscript{2+} concentration in all uptake media, 0.2 μM tetra K\textsuperscript{+} form of Fura 2 was added to a sample of medium maintained at 37 °C. Increasing concentrations of EGTA were added and free Ca\textsuperscript{2+} was calculated from F, F\textsubscript{min}, and F\textsubscript{max} (35). Addition of 40 μM EGTA to the uptake medium resulted in an ionized Ca\textsuperscript{2+} concentration of 248 nM. Acini suspended in uptake medium were kept on ice for 30–60 min before permeabilization. The acini were permeabilized by two exposures to an electric field of 0.75 kV through a capacitance of 250pF using a Gene Pulser (Bio-Rad). Cell permeabilization was then estimated from the measurements of stibium bromide fluorescence as previously described (37, 38). Routinely, more than 90% of the cells were permeabilized by the applied electric field. To initiate Ca\textsuperscript{2+} uptake, the permeabilized acini were added to uptake medium also containing the following (final concentrations): antimycin A, 10 μM; oligomycin, 5 μM; 46Ca (approximately 10\textsuperscript{5} cpm/ml), with or without 2 mM ATP and 10 mM PGA as specified. The pH of all solutions was verified to be 7.2 at 37 °C prior to addition of "Ca and initiation of uptake. At the indicated times, 0.2 ml of cell suspension was transferred to 8 ml of ice-cold uptake medium containing 1 mM LaCl\textsubscript{3}. The acini were washed three times with this medium and dissolved in 1 ml of 1 M NaOH, and 46Ca was counted.

**RESULTS**

The duration of [Ca\textsuperscript{2+}], increase at two hormone concentrations is shown in Fig. 1. Stimulation of acini with 2.5 μM carbachol resulted in an increase in [Ca\textsuperscript{2+}], to 342 nM within approximately 2.5 s [Ca\textsuperscript{2+}], remained at this level for about 12–15 s and was then reduced to near-resting levels (Fig. 1a). When acini were stimulated with 200 μM carbachol, [Ca\textsuperscript{2+}], was increased to 1129 nM within 4 s, followed by a reduction in [Ca\textsuperscript{2+}], to near-resting levels (Fig. 1b). At all submaximal hormone concentrations tested, the increase in [Ca\textsuperscript{2+}], occurred during the first 2.5–4 s of stimulation, after which [Ca\textsuperscript{2+}], remained constant for approximately 12–15 s. The short period of [Ca\textsuperscript{2+}], increase can result from the following events.

(a) Ca\textsuperscript{2+} release from internal stores continues for the period of stimulation until these stores are depleted of Ca\textsuperscript{2+}. The internal stores and the plasma membrane Ca\textsuperscript{2+} pumps are stimulated within 2.5–4 s of stimulation. In the subsequent 12–15 s, the pumps remove Ca\textsuperscript{2+} from the cytosol at the same rate at which Ca\textsuperscript{2+} is released from the internal stores; thus the pumps reduce [Ca\textsuperscript{2+}], to near-resting levels. Thus, Ca\textsuperscript{2+} release from internal stores is balanced by Ca\textsuperscript{2+} pumping out of the cytosol while Ca\textsuperscript{2+} is continuously released from internal stores.

(b) The internal Ca\textsuperscript{2+} stores became refractory to Ins-1,4,5-P\textsubscript{3} and perhaps IcP3 after 2.5–4 s of stimulation so that Ca\textsuperscript{2+} release from internal stores ceased after short periods of stimulation.

(c) The internal Ca\textsuperscript{2+} stores are compartmentalized and at increasing hormone concentrations an increasing fraction of internal Ca\textsuperscript{2+} is released. Thus, the hormone-mediated Ca\textsuperscript{2+} release from internal stores is a quantal process. The results presented below support the third possibility.

- Fig. 2 shows the effect of a maximal dose of carbachol or CCK-OP on [Ca\textsuperscript{2+}], of cells prestimulated for 4 min with different concentrations of carbachol. As expected, the initial
[Ca^{2+}], increase was a function of carbachol concentrations between 2.5 and 200 μM. Furthermore, a subsequent maximal dose of carbachol or CCK-OP gives a submaximal increase in [Ca^{2+}], which is inversely proportional to the initial carbachol concentration. The effect of reducing medium Ca^{2+} to 10 μM on this pattern of hormonally evoked [Ca^{2+}], increase is recorded in Fig. 3. Reducing medium Ca^{2+} had only a small effect on the initial carbachol-stimulated [Ca^{2+}], increase and did not prevent the [Ca^{2+}], increase evoked by a subsequent stimulation with a maximal hormone concentration. The experiments in Fig. 2 and 3 indicate that carbachol and CCK-OP release Ca^{2+} from the same intracellular stores. The finding that, in the presence and absence of medium Ca^{2+}, the maximal doses of CCK-OP or carbachol could still increase [Ca^{2+}], when added to acini prestimulated with any submaximal dose of carbachol clearly shows that a submaximal dose of the hormones did not release all the Ca^{2+} from internal stores.

The Ca^{2+} content of intracellular stores after acini stimulation with submaximal concentrations of hormones for different periods of time is shown in Fig. 4 and Table I. For these experiments, acini were stimulated with 2.5 μM carbachol (Fig. 4, a-d) or 0.2 nM CCK-OP (Fig. 4, e-h). After 1, 5, 10, or 15 min of stimulation, the acini were exposed to 200 μM carbachol or 10 nM CCK-OP, respectively. As can be seen in Fig. 4, a, b, e, f, the maximal concentration of the hormones increased [Ca^{2+}], to approximately the same level after 1 or 5 min of stimulation with a submaximal dose of the hormones. When the acini were stimulated for longer periods of time, the maximal dose of hormones increased [Ca^{2+}], to somewhat lower levels. Table I summarizes the results of three experiments in which the acini were stimulated with different concentrations of carbachol or CCK-OP for 5, 10, and 15 min prior to exposure of the acini to a maximal dose of hormone. The results show that the pattern of [Ca^{2+}], increase shown in Fig. 4 applies to all doses of submaximal hormone concentrations tested. Thus, it appears that, at all hormone concent-
tations, the rapid release of Ca\(^{2+}\) from intracellular stores occurs at the first few seconds of stimulation.

To demonstrate the effect of continuous Ca\(^{2+}\) release from intracellular stores on the ability of a maximal dose of hormone to increase [Ca\(^{2+}\)], we tested the effect of two concentrations of the Ca\(^{2+}\) ionophore ionomycin on [Ca\(^{2+}\)]. Fig. 5 shows that stimulation of acini with 10 nM CCK-OP increased [Ca\(^{2+}\)], to approximately 1061 nM. When the acini were incubated with 20 nM ionomycin for 2, 5, or 10 min prior to stimulation with CCK-OP, the hormone increased [Ca\(^{2+}\)], to 427, 269, and 191 nM, respectively. Exposure of the acini to 2.5 nM ionomycin had a very small effect on [Ca\(^{2+}\)] (Fig. 5e). However, incubation of the acini with 2.5 nM ionomycin for 5, 10, or 15 min resulted in a time-dependent reduction in the ability of CCK-OP to increase [Ca\(^{2+}\)]. Although the concentrations of ionomycin used had a small effect on [Ca\(^{2+}\)], they depleted the intracellular stores of Ca\(^{2+}\) in a time-stepped manner, since the ionophore continuously releases Ca\(^{2+}\) from internal stores. This experiment also shows that most of the initial, hormone-evoked increase in [Ca\(^{2+}\)], is due to Ca\(^{2+}\) release from intracellular stores. Incubation of the acini with 20 nM ionomycin for 10 min almost completely prevented the ability of CCK-OP to increase [Ca\(^{2+}\)]. Since the incubation with ionomycin should have no effect on Ca\(^{2+}\) entry across the plasma membrane, the diminution of the effect of CCK-OP on [Ca\(^{2+}\)] was therefore due to the depletion of the intracellular stores by ionomycin.

To demonstrate that the intracellular stores remain partially depleted of Ca\(^{2+}\) during stimulation with submaximal doses of hormone, we tested the effect of atropine on the ability of CCK-OP to increase [Ca\(^{2+}\)], in carbachol-treated acini (Fig. 6). Stimulation of acini with 5 μM carbachol increased [Ca\(^{2+}\)], to 413 nM. Subsequent stimulation with 10 nM CCK-OP increased [Ca\(^{2+}\)], to only 544 nM (Fig. 6a). When carbachol-treated acini were incubated with atropine for 5 min prior to stimulation with CCK-OP, CCK-OP increased [Ca\(^{2+}\)], to 1203 nM (Fig. 6b), a level similar to that induced by stimulation of untreated acini (control) with CCK-OP. These experiments, together with \(^{41}\)Ca uptake and efflux measurements from carbachol-stimulated cells (9-12, 28, 29, 36), indicate that the Ca\(^{2+}\) permeability of the fraction of internal stores releasing Ca\(^{2+}\) remains elevated for as long as the cells are stimulated.

**Fig. 5. Effect of ionomycin on intracellular Ca\(^{2+}\) stores.** Fura-2-loaded acini were suspended in albumin-free solution A to prevent binding of the ionophore by albumin. Acini were stimulated with 10 nM CCK-OP before (a) or after incubation with ionomycin (b-g). Acini were incubated with 20 nM ionomycin for 2 (b), 5 (c), or 10 min (d) or with 2.5 nM ionomycin for 5 (e), 10 (f), or 15 min (g) before stimulation with 10 nM CCK-OP. Identical results were obtained when the acini were stimulated with 200 μM carbachol. The figure shows six (b-d, e-g) separate, superimposed experiments.

**Fig. 6. Termination of cell stimulation is required for Ca\(^{2+}\) reloading.** Acini suspended in solution A containing 2 mM CaCl\(_2\) were stimulated with 5 μM carbachol and then with 10 nM CCK-OP as indicated (a). Acini stimulated with 5 μM carbachol were incubated with 20 μM atropine for 5 min (b) prior to stimulation with CCK-OP. The experiment shown represents two others with similar results.

**Fig. 7. CCK-OP-mediated \(^{41}\)Ca release from intracellular stores.** Intracellular stores were labeled with \(^{41}\)Ca to isotopic equilibrium as described under "Experimental Procedures." After the \(^{41}\)Ca labeling the acini were suspended in solution A containing 2 mM unlabeled Ca\(^{2+}\). After 5 min of incubation at 37°C, 4-ml portions were transferred to tubes containing CCK-OP to yield the following concentrations: 0.0 (control, ◆), 0.05 (Δ), 0.2 (◆), 1.0 (□), and 10 nM (O). After 15 min of stimulation with the indicated concentrations of CCK-OP, all acini were exposed to 10 mM CaCl\(_2\) for 5 min. At the indicated times, samples of acini were removed to determine \(^{41}\)Ca content, as described under "Experimental Procedures." The figure shows the mean ± S.D. of four experiments.

Measurements of [Ca\(^{2+}\)], reflect the sum of the activities of at least four Ca\(^{2+}\) transporting pathways: conductive pathways in the plasma and internal stores membranes which increase [Ca\(^{2+}\)], and the plasma and internal stores Ca\(^{2+}\) pumps which remove Ca\(^{2+}\) from the cytosol. The ability of a second addition of a maximal dose of hormone to increase [Ca\(^{2+}\)], may be due in part to reuptake of the released Ca\(^{2+}\) back into the internal stores. In addition, a small reduction in the ability of the second addition of hormone to increase [Ca\(^{2+}\)], was observed when acini were stimulated with a submaximal dose of hormone for extended periods of time (Table 1). Therefore, to demonstrate the quantal feature of Ca\(^{2+}\) release from internal stores, it was necessary to separate this process from the activity of other Ca\(^{2+}\) transport pathways. To achieve this, the effect of different CCK-OP concentrations on \(^{41}\)Ca efflux was measured (Fig. 7). The intracellular Ca\(^{2+}\) pool was first labeled to isotopic equilibrium with \(^{41}\)Ca (see "Experimental Procedures.") Stimulation of these acini with increasing CCK-
OP concentrations resulted in an increased fraction of Ca²⁺ release from the cells. No submaximal hormone concentration was able to release all the Ca²⁺ from intracellular stores during the 15 min of cell stimulation. At each hormone concentration the cells lost Ca²⁺ only during the first 5 min, after which the cellular Ca²⁺ level remained constant. The ⁴⁰Ca efflux measurements in Fig. 7 represent exclusively Ca²⁺ release from the pool. In case submaximal hormone concentrations premeabilize the entire intracellular Ca²⁺ pool, but the pool is only partially depleted of Ca²⁺ due to reuptake of Ca²⁺ into the pool, then submaximal hormone concentrations should release all the ⁴⁰Ca from the cells since under the conditions of Fig. 7, ⁴⁰Ca is released, whereas only ⁴⁰Ca is available for Ca²⁺ reuptake. Thus, the findings in Fig. 7 clearly exclude the possibility that reuptake of Ca²⁺ into the pool is responsible for the partial depletion of the pool by submaximal hormone concentrations.

To compare the quantal kinetic properties of the hormone-mediated Ca²⁺ release from internal stores to that of a continuous, nonselective Ca²⁺ release, the effect of different concentrations of ionomycin on cellular Ca²⁺ content was measured. Fig. 8 shows that increasing ionomycin concentrations from 2.5 to 250 nM resulted in increased rate of Ca²⁺ release from internal stores. More importantly, each ionomycin concentration eventually completely depleted all the internally stored Ca²⁺. Thus, with increasing ionomycin concentrations only the rate of Ca²⁺ release was increased but all the stored Ca²⁺ could be mobilized. In contrast, with increasing hormone concentrations the fraction of mobilizable Ca²⁺ increased (Fig. 7).

The hormone-dependent Ca²⁺ release from intracellular stores involves the generation of Ins-1,4,5-P₃ in the cytosol which subsequently mediates the Ca²⁺ release from intracellular stores (17, 18). Therefore, it was of interest to study the kinetic properties of Ins-1,4,5-P₃-mediated Ca²⁺ release from the internal stores of pancreatic acinar cells. The difficulty in this type of study is that permeabilized cells can hydrolyze Ins-1,4,5-P₃. However, Ins-1,4,5-P₃ hydrolysis can be inhibited by PGA (39). Fig. 9 shows the effect of 10 mM PGA on Ins-1,4,5-P₃-mediated Ca²⁺ release from electrically permeabilized pancreatic acini. Including 10 mM PGA in the uptake medium had only a small effect on the rate or extent of ATP-dependent Ca²⁺ uptake into nonmitochondrial, intracellular Ca²⁺ stores. Addition of Ins-1,4,5-P₃ to acini incubated in the absence of PGA resulted in a transient release of Ca²⁺. However, in the presence of PGA, Ins-1,4,5-P₃ induced the release of approximately 1.6 nmol of Ca²⁺/mg of protein and the stores did not reload with Ca²⁺. This experiment indicates that, in pancreatic acinar cells, PGA was effective in preventing Ins-1,4,5-P₃ hydrolysis, thus resulting in a maintained depletion of the internal Ca²⁺ stores by Ins-1,4,5-P₃.

Next, the protocol in Fig. 9 was used to test the effect of different Ins-1,4,5-P₃ concentrations on Ca²⁺ release from

**Fig. 8. Ionomycin-mediated Ca²⁺ release from intracellular stores.** Experimental procedure was identical to that described in the legend to Fig. 7 except for the following: The ⁴⁰Ca-labeled acini were suspended in albumin-free solution A and then transferred to tubes containing ionomycin to yield the following concentrations: 0.0 (control, A), 2.5 (A), 10 (A), 50 (C), and 250 nM (C). At the indicated times acini were removed to the stop solution containing 1 mM LaCl₃ and 25 mg/ml albumin. Albumin was included in the stop solution to scavenge the ionophore. The figure shows the mean ± S.D. of four experiments.

**Fig. 9. Effect of PGA on Ins-1,4,5-P₃-mediated Ca²⁺ release.** Acini were permeabilized and Ca²⁺ uptake was measured as described in detail under "Experimental Procedures." The uptake medium was with (□, ◦) or without (□, □) 2 mM ATP and with (□, ■) or without (□, □) 10 mM PGA. After 4 min of incubation at 37 °C in the presence of ATP, samples of acini were transferred to tubes containing Ins-1,4,5-P₃ to yield a final concentration of 5 μM (∆, Δ). At the indicated times, samples were removed to measure ⁴⁰Ca content. The experiment shown is one of three experiments with similar results.

**Fig. 10. Properties of Ins-1,4,5-P₃-mediated Ca²⁺ release.** Ca²⁺ uptake and release from permeabilized pancreatic acini were measured as described under "Experimental Procedures." The uptake medium contained 2 mM ATP and 10 mM PGA. After 2 min of incubation at 37 °C, acini were transferred to tubes containing Ins-1,4,5-P₃ to yield the following concentrations: 0.0 (control, ◦), 0.25 (△), 0.5 (□), 1.0 (◆), 2.5 (◇), or 5.0 (◇) μM (∆). At the indicated times, samples were removed to measure ⁴⁰Ca content of acini. The experiment shown represents four experiments with similar results.
mediator. Furthermore, each concentration of the mediator pump inhibition was much slower than the rate of Ins-1,4,5-
membrane (19, 20), which results in Ca\textsuperscript{2+} release from this pool activating a Ca\textsuperscript{2+} conductive pathway in the internal pool mem-
the Ins-1,4,5-P\textsubscript{3}-evoked Ca\textsuperscript{2+} release is the result of pump-
the cytosol. In the present studies we attempted to increase in parallel with increasing levels of the Ca\textsuperscript{2+} releasing
mation shown represents three experiments with similar results.

internal stores. Fig. 10 shows the quantal nature of this process. Increasing Ins-1,4,5-P\textsubscript{3} concentrations resulted in increasing fractions of Ca\textsuperscript{2+} release. Again, no submaximal Ins-1,4,5-P\textsubscript{3} concentration was capable of releasing all the Ca\textsuperscript{2+} from the internal stores. With increasing Ins-1,4,5-P\textsubscript{3} concentrations, larger fractions of the internal Ca\textsuperscript{2+} store were susceptible to Ins-1,4,5-P\textsubscript{3}-mediated Ca\textsuperscript{2+} release.

Again, to exclude the possibility that the quantal feature of the Ins-1,4,5-P\textsubscript{3}-evoked Ca\textsuperscript{2+} release is the result of pump-leak turnover across the pool membrane, we tested the effect of pump inhibition on Ins-1,4,5-P\textsubscript{3}-evoked Ca\textsuperscript{2+} release. Fig. 11 shows that addition of VO\textsubscript{4}\textsuperscript{2-}, at a concentration which was sufficient to completely inhibit Ca\textsuperscript{2+} uptake (38), resulted in Ca\textsuperscript{2+} efflux from the acini. The rate of Ca\textsuperscript{2+} efflux due to pump inhibition was much slower than the rate of Ins-1,4,5-
P\textsubscript{3}-mediated Ca\textsuperscript{2+} release. Furthermore, pump inhibition did not result in augmentation of Ca\textsuperscript{2+} release induced by 0.5 or 5 \textmu M Ins-1,4,5-P\textsubscript{3} during the first 1 min of incubation. Ins-
1,4,5-P\textsubscript{3}-mediated Ca\textsuperscript{2+} release is completed within less than 30 s. Therefore, it appears that Ins-1,4,5-P\textsubscript{3}-evoked Ca\textsuperscript{2+} release is a quantal process, similar to that found for hormone-evoked Ca\textsuperscript{2+} release from intracellular stores.

DISCUSSION

Stimulation of cells with Ca\textsuperscript{2+}-mobilizing hormones induces a transient [Ca\textsuperscript{2+}], increase. The hormones mobilize Ca\textsuperscript{2+} from extracellular and intracellular pools (2-4, 6-8). The initial, hormone-evoked [Ca\textsuperscript{2+}], increase is largely due to Ca\textsuperscript{2+} release from intracellular stores (1, 2, 5, 7, 9-16). The latter process is widely believed to involve the generation of Ins-1,4,5-P\textsubscript{3} in the cytosol (17, 18). Ins-1,4,5-P\textsubscript{3}, in turn, is capable of activating a Ca\textsuperscript{2+} conductive pathway in the internal pool mem-
brane (19, 20), which results in Ca\textsuperscript{2+} release from this pool into the cytosol. In the present studies we attempted to determine whether the Ca\textsuperscript{2+} release from intracellular stores is a continuous or a quantal process. For a continuous process to occur, the rate of Ca\textsuperscript{2+} release from intracellular stores must be capable of completely depleting the internal stores of Ca\textsuperscript{2+}. Thus, measurements of the time course of Ca\textsuperscript{2+} release from intracellular stores at different concentrations of the mediator must result in a family of single exponential Ca\textsuperscript{2+} efflux curves, each of which has different rate constant. This was the type of kinetic behavior observed when the effect of the Ca\textsuperscript{2+} ionophore ionomycin on [Ca\textsuperscript{2+}], (Fig. 6) or Ca\textsuperscript{2+} content in the intracellular stores (Fig. 8) was measured. In a quantal process, when the levels of the Ca\textsuperscript{2+} releasing mediator are increased, increasing fractions of the Ca\textsuperscript{2+} stored in the intracellular pools are released. At each mediator concentra-
tion only part of the internally stored Ca\textsuperscript{2+} is released and no submaximal mediator concentration should release all the Ca\textsuperscript{2+} from the internal stores. This process gives rise to an "all or none" behavior in terms of Ca\textsuperscript{2+} release from the stores. Hence, at a given submaximal mediator concentration, all the Ca\textsuperscript{2+} is released from a fraction of the stores, whereas none of the Ca\textsuperscript{2+} is released from the remaining, nonresponding, fraction.

The hormone-mediated Ca\textsuperscript{2+} release from intracellular stores was expected to be a continuous rather than a quantal process. Measurements of the time course of hormonal-induced Ins-1,4,5-P\textsubscript{3} generation in pancreatic acinar (25, 30) and other cell types (22, 31, 32) shows that the cellular concentration of Ins-1,4,5-P\textsubscript{3} continues to increase for approximately 10 s following cell stimulation. In the subsequent 1-2 min of cell stimulation, Ins-1,4,5-P\textsubscript{3} levels decline to about half their maximum and are then maintained at the elevated levels for at least 20 min of cell stimulation (25). This time course implies that, during the entire stimulation period, the internal Ca\textsuperscript{2+} stores membrane is exposed to high concentrations of Ins-1,4,5-P\textsubscript{3} and therefore should consequently remain permeable to Ca\textsuperscript{2+} for as long as the cells are stimulated.

Indeed, 4\textsuperscript{5}Ca influx (9-12, 28, 30) and efflux (29) measurements from cells stimulated with a maximal dose of hormone confirm this and show that the Ca\textsuperscript{2+} permeability of the internal pool membrane remains elevated during the entire stimulation period. Stimulation of pancreatic acini with Ca\textsuperscript{2+}-mobilizing hormones dose-dependently increased the rate of phosphatidylinositol 4,5-bisphosphate hydrolysis, the precursor of Ins-1,4,5-P\textsubscript{3} (40). It is, therefore, likely that with increasing hormone concentrations, the levels of Ins-1,4,5-P\textsubscript{3} in the cytosol increase in parallel. If the internal stores do not become refractory to Ins-1,4,5-P\textsubscript{3} during the stimulation period, the maintained levels of Ins-1,4,5-P\textsubscript{3} in the cytosol predict that at each hormone concentration, the intracellular stores should be completely depleted of Ca\textsuperscript{2+} and Ca\textsuperscript{2+} release should be a continuous process.

Examination of the properties of the hormone-mediated Ca\textsuperscript{2+} release from intracellular stores shows this process to be quantal rather than continuous. [Ca\textsuperscript{2+}], and 4\textsuperscript{5}Ca efflux measure-
ments were used to provide evidence for such behavior. When the cells were stimulated with different concentrations of hormones, [Ca\textsuperscript{2+}], increased only during the first 2-4 s of stimulation. This was not due to hydrolysis of Ins-1,4,5-P\textsubscript{3}, since the levels of Ins-1,4,5-P\textsubscript{3} remain elevated for longer periods of time (25, 30). It is equally unlikely that the internal stores became refractory to Ins-1,4,5-P\textsubscript{3}. If this was to occur, the Ca\textsuperscript{2+} permeability of the pool membrane would have been reduced to that of unstimulated cells. Under these conditions, the internal stores can spontaneously and completely reload with Ca\textsuperscript{2+}. That this was not the case was shown in Fig. 6, in which incubation of carbachol-stimulated acini with atropine was necessary for Ca\textsuperscript{2+} reloading and recovery of maximal CCK-OP response. The requirement for incubation with atro-
pine at higher carbachol concentrations for reloading to take place was previously demonstrated (7, 12). Thus, the findings that Ca\textsuperscript{2+} reloading at all hormone concentrations required inhibition of cell stimulation strongly suggest that elevated
Ins-1,4,5-P_3 levels are maintained for the duration of cell stimulation and the internal stores membrane does not become refractory to Ins-1,4,5-P_3 during the stimulation period. Stimulation of Ca^{2+} efflux out of the cells which offset continuous Ca^{2+} release from intracellular stores also cannot explain the observed short period of increase in [Ca^{2+}]. In this case, intracellular stores should be depleted of Ca^{2+} within less than 1 min. However, maximal doses of hormones added to cells stimulated with submaximal hormone concentrations increased [Ca^{2+}] to similar levels even after 15 min of stimulation. In addition, [Ca^{2+}] increase by the maximal dose of hormone was inversely related to the initial [Ca^{2+}]; increase induced by the submaximal hormone concentrations (Figs. 2 and 3). These findings suggest that net Ca^{2+} release from intracellular stores occurs only during the first 2.5-4 s of cell stimulation.

To explain the short and limited Ca^{2+} release, it is necessary to suggest that the hormone-evoked Ca^{2+} release from intracellular stores is a quantal process. This is supported by the findings in Table I and Fig. 7. For each submaximal hormone concentration, only a part of the Ca^{2+} was released to the cytosol. This Ca^{2+} was then pumped out of the cytosol as reflected by the reduction in [Ca^{2+}]. (Fig. 3) and "Ca efflux (Fig. 7). Ca^{2+} content of the remaining stores remained nearly constant for the duration of cell stimulation and was available for release by a maximal dose of hormone. Thus, each dose of hormone releases only a fraction of the stored Ca^{2+}. Increasing hormone concentrations increased the fraction of intracellular Ca^{2+} released. That this phenomenon does not reflect Ca^{2+} permeabilization of the entire intracellular Ca^{2+} pool membranes by submaximal hormone concentrations and Ca^{2+} reuptake into the pool by the pool Ca^{2+} pump is concluded from the "Ca efflux measurements (Fig. 7). The findings that at all submaximal hormone concentrations only part of the "Ca could be released or exchanged with medium and cytosolic Ca^{2+} excludes the possibility that submaximal hormone concentrations permeabilize the membranes of the entire intracellular pool to Ca^{2+}.

Since Ins-1,4,5-P_3-P_3 is believed to be the mediator of Ca^{2+} release from internal stores (17, 18), we tested the pattern of Ins-1,4,5-P_3-triggered Ca^{2+} release. For these experiments permeabilized cells rather than isolated vesicles were used to preserve the structural organization of the internal stores. The pattern of Ins-1,4,5-P_3-triggered Ca^{2+} release from intracellular stores was similar to that observed with the hormones. Increasing Ins-1,4,5-P_3 concentrations between 0.25 and 2.5 μM resulted in increased fractions of Ca^{2+} release from internal stores. This pattern of Ins-1,4,5-P_3-evoked Ca^{2+} release was observed also when Ca^{2+} uptake was inhibited during the period of Ca^{2+} release. Hence, the quantal properties of hormone-evoked Ca^{2+} release are likely to stem from the mode by which Ins-1,4,5-P_3 releases the Ca^{2+}.

A minimal requirement for obtaining a quantal response is that the internal Ca^{2+} stores be extensively compartmentalized. The nature of the intracellular Ca^{2+} storage pool is not clear at present. Many studies have implicated the endoplasmic reticulum or a fraction thereof (for review, see Ref. 18) as the organelle storing the hormone-mobilizable Ca^{2+} pool. However, recently, an intracellular organelle which contains a Ca^{2+}-binding protein related to calsequestrin (41) was identified in HL-60, PC 12, liver, and pancreatic acinar cells (42). This organelle, which was termed "calciosome," is capable of accumulating Ca^{2+} and may represent the Ins-1,4,5-P_3-sensitive Ca^{2+} pool in nonmuscle cells. The calciosomes appear to be separated from each other (42) and therefore can respond individually to a second messenger. It appears, therefore, that the calciosomes, or an equivalent structural arrangement of intracellular Ca^{2+} stores, can provide the requirement for compartmentalization of the intracellular stores.

Another requirement for obtaining a quantal response is the compartmentalization of the Ca^{2+} release process. This can be achieved in at least two ways. It is possible that the generation of Ins-1,4,5-P_3 is localized, and due to its charge, Ins-1,4,5-P_3 does not diffuse in the cytosol but remains confined to its site of generation. With increasing hormone concentrations, Ins-1,4,5-P_3 is generated in additional parts of the cytosol, which leads to Ca^{2+} depletion from increasing fractions of the stores. Alternatively, the compartmentalized stores may have different sensitivities to Ins-1,4,5-P_3, and they respond to Ins-1,4,5-P_3 in an "all or none" manner. Thus, individual compartments of these stores exhibit different thresholds for Ins-1,4,5-P_3. With increasing hormone concentrations, the concentration of Ins-1,4,5-P_3 in the cytosol is increased and increasing fractions of the stores become susceptible to Ins-1,4,5-P_3-mediated Ca^{2+} release. Although it is difficult to distinguish between these possibilities, the pattern of Ins-1,4,5-P_3-mediated Ca^{2+} release from permeabilized cells (Figs. 10 and 11) appears to support the second possibility. In permeabilized cells the entire intracellular pool is exposed to Ins-1,4,5-P_3. Nonetheless, submaximal concentrations of Ins-1,4,5-P_3 did not release all the Ca^{2+} from internal stores. Such behavior does not necessitate that each Ins-1,4,5-P_3-activated Ca^{2+} channel (19, 20) operate in an "all or none" fashion, (ie. it can be either in an open or closed state). It is sufficient that the channels display different sensitivities or threshold for activation by Ins-1,4,5-P_3.

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REFERENCES
Quantal Properties of Ca\textsuperscript{2+} Release